Nucleus basalis of Meynert degeneration precedes and predicts cognitive impairment in Parkinson's disease

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Abstract

Currently, no reliable predictors of cognitive impairment in Parkinson's disease exist. We hypothesised that microstructural changes at grey matter T1-weighted MRI and diffusion tensor imaging in the cholinergic system nuclei and associated limbic pathways underline cognitive impairment in Parkinson's disease. We performed a cross-sectional comparison between Parkinson's disease patients with and without cognitive impairment. We also performed a longitudinal 36-month follow up study of cognitively intact Parkinson's patients, comparing patients who remained cognitively intact to those who developed cognitive impairment. Parkinson's disease patients with cognitive impairment showed lower grey matter volume and increased mean diffusivity in the nucleus basalis of Meynert, compared to Parkinson's disease patients without cognitive impairment. These results were confirmed both with region-of-interest and voxel-based analyses, and after partial volume correction. Lower grey matter volume and increased mean diffusivity in the nucleus basalis of Meynert was predictive for developing cognitive impairment in cognitively intact patients with Parkinson's disease, independent of other clinical and non-clinical markers of the disease. Structural and microstructural alterations in entorhinal cortex, amygdala, hippocampus, insula, and thalamus were not predictive for developing cognitive impairment in Parkinson's disease. Our findings provide evidence that degeneration of the nucleus basalis of Meynert precedes and predicts the onset of cognitive impairment, and might be used in clinical setting as reliable biomarker to stratify patients at higher risk of cognitive decline.

Keywords: nucleus basalis of Meynert; MRI; DTI; cognitive decline; Parkinson's disease

Introduction

When James Parkinson first described the "shaking palsy" in 1871, he assumed that "the senses and intellect were uninjured" (Parkinson, 1817). Unfortunately, this claim was not fully accurate (Saeed *et al.*, 2017). Cognitive impairment is highly prevalent in Parkinson's disease, and approximately 80% of Parkinson's disease patients will eventually develop dementia during the course of their illness (Hely *et al.*, 2008). Cognitive impairment is one of the most clinically relevant symptoms in Parkinson's disease (Aarsland *et al.*, 2012) and causes an increased risk of mortality and significant reduction in quality of life (Forsaa *et al.*, 2010; Winter *et al.*, 2011).

The mechanisms underlying the development of cognitive impairment in Parkinson's disease remain unclear. Several imaging and clinical markers have been evaluated over the past years as potential predictors for the development of cognitive impairment in Parkinson's disease (Moore and Barker, 2014; Xu *et al.*, 2016). Clinical markers for predicting cognitive impairment in Parkinson's disease vary across studies with contradicting evidence. Depression, REM sleep behaviour disorder (Aarsland *et al.*, 2003; Zhu *et al.*, 2014) gait dysfunction, cerebrovascular diseases associated with white matter lesions (Ma *et al.*, 2015), olfactory dysfunction, Apo-E genotype, and the ratio CSF Amyloid-β:Tau (Schrag *et al.*, 2017) have been suggested as predictors for the development of cognitive impairment in Parkinson's disease (Aarsland *et al.*, 2003), however, several of these have been disputed (Zhu *et al.*, 2014). Imaging studies have shown cortical and subcortical brain regions to be predictive of cognitive impairment in Parkinson's disease, including structural and microstructural changes within the entorhinal cortex, amygdala, hippocampus, insula, thalamus, striatum and tempo-parieto-frontal areas (Hattori *et al.*, 2012; Melzer *et al.*, 2012).

However, evidence from these studies are limited due to small sample sizes, caveats in study designs, classification of cognitive impairment, and subject inclusion criteria.

Loss of cholinergic innervation of the cerebral cortex has been suggested as one mechanism of dementia (Aarsland *et al.*, 2017) and pathological changes seen in Parkinson's disease patients with cognitive impairment support this theory. In the basal forebrain, extra-nigral Lewy bodies are present in neurons of the nucleus basalis of Meynert, the primary source of cholinergic innervation of the cerebral cortex (Bohnen and Albin, 2011a). Braak *et al.* suggests this cholinergic neuron degeneration occurs at the same stage as nigral pathology (Braak *et al.*, 2003). Imaging studies have demonstrated structural and microstructural changes within the nucleus basalis of Meynert to be predictive of cognitive impairment (Lee *et al.*, 2014; Schmitz and Nathan Spreng, 2016). Significant loss of cholinergic neurons in the nucleus basalis of Meynert has been observed in Parkinson's disease in the absence of Alzheimer's disease pathology (Rogers *et al.*, 1985), and positron emission tomography imaging studies have confirmed that cholinergic neurons in the basal forebrain degenerates at early stages of Parkinson's disease. This degeneration progresses with the onset of cognitive impairment (Bohnen and Albin, 2011a). Lower levels of choline acetyltransferase and acetylcholinesterase have also been associated with cognitive impairment in Parkinson's disease, at similar levels as seen in Alzheimer's disease (Bohnen *et al.*, 2003). Currently, no robust predictors of cognitive impairment are validated and used in clinical practice.

Here, we hypothesised that structural and microstructural changes in the cholinergic system nuclei and associated limbic pathways could be underlying cognitive impairment in patients with Parkinson's disease, and moreover could predict the development of cognitive impairment. We sought to investigate this hypothesis by performing a cross-sectional

baseline comparison of MRI data between Parkinson's disease patients with and without cognitive impairment, and a longitudinal 36-month comparison of MRI data between those Parkinson's patients who remained cognitively intact and those who developed cognitive impairment (Figure 1).

Methods

Study participants. Data used for this paper were obtained from the Parkinson's Progression Marker Initiative (PPMI) database (www.ppmi-info.org/data) in January 2017. We have excluded subjects with a history of stroke or transient ischemic attack. A total of 304 Parkinson's disease patients not on Parkinson's medications (drug-naïve) and 167 heathy controls were identified. All patients underwent an initial screening visit followed by a baseline visit where demographics, family history, clinical characteristics, cognitive status and non-motor symptoms measurements were collected (Supplementary Methods).

Cognitive assessments. Cognitive status was assessed at baseline and follow-up visits every 6 months (terminated at 36 months, or earlier if a patient developed cognitive impairment). Cognitive function was defined as two levels. Level 1 diagnosis was done on all patients at baseline and was determined based on MoCA scores. Patients with MoCA ≥26 were screened as cognitively normal (PD-MoCA≥26; n=232), and patients with MoCA ≤25 were screened as with cognitive impairment (PD-MoCA≤25; n=72). Level 2 diagnosis was determined at follow-up visits with MoCA≤25, self-reported issues in cognitive function, and at least two cognitive test scores (irrespective of test domain) greater than 1.5 standard deviations (SD) below mean healthy control age/education standardized scores as published previously (Weintraub *et al.*, 2015). At the end of the 36-month period, 35 Parkinson's patients satisfied Level 2 diagnosis for cognitive impairment, whereas 197 patients did not .

Image acquisition and processing. T1weighted (T1-MRI) and T2-weighted MRI (T2-MRI) images were acquired by Philips, GE, or SIEMENS machines with either 1.5T or 3T strength. Diffusion tensor imaging images were obtained with SIEMENS machines using a 2D single-shot echo planar imaging sequence with 3T strength (Supplementary Methods). T1-MRI images were pre-processed using Statistical Parametric Mapping 12 (SPM, Wellcome Trust Centre for Neuroimaging, London, UK) implemented in MATLAB (The Math-Works, Natick, MA, USA) to allow for grey matter voxel based morphometry analysis. Images were segmented and modulated into grey matter, white matter, CSF, bone and soft tissue. The grey matter image was smoothened, to cope with functional anatomical variability, and normalized and aligned into Montreal Neurological Institute (MNI) space. The final T1-MRI image map represents the volume of grey matter within each voxel. T2-MRI images were co-registered with T1-MRI for each subject to rule out vascular pathology and quantify the volume of white matter lesions.

Diffusion weighting was isotropically distributed along 64 gradient directions with a b-value of 1000s/mm^2, and a non-diffusion-weighted imaging (b0) was acquired at the start of each scan. Diffusion data analysis was performed with FSL Diffusion Toolbox (FDT) (FMRIB Software Library (FSL), Centre Software Library, University of Oxford, Oxford, UK); topup (Andersson *et al.*, 2003) and eddycorrect (Andersson and Sotiropoulos, 2016) corrected for head motion, artefacts and eddy currents. DTIFit fitted diffusor tensor model maps at each voxel to generate fractional anisotropy maps, and mean diffusivity maps (Supplementary Methods).

Grey matter analysis was done by T1-MRI images obtained from all patients described (Figure 1A). Diffusion tensor imaging analysis was done on a subset of 62 healthy control and 84 Parkinson's disease patients (Figure 1B). Of the Parkinson's disease patients, 64 were screened as not cognitively impaired at baseline (PD-MoCA≥26) and 20 were screened as cognitively impaired at baseline (PD-MoCA≤25). Patients not cognitively impaired at baseline screening were followed up for the same period: 17 Parkinson's disease patients satisfied Level 2 diagnosis for cognitive impairment, whereas 47 patients did not.

To account for the variability across MRI scanners, we investigated differences in grey matter mean voxel values obtained by different MRI manufacturers (Philips vs. GE. vs. SIEMENS) and with different strength of field (1.5 vs. 3T). We also investigated differences in diffusion tensor imaging mean diffusivity mean voxel values obtained with different protocols (gated vs. non-gated). Considering that the variability in diffusion tensor imaging across cameras is usually high, we investigated the cross-centre variance of mean diffusivity mean voxel values.

Regions-of-interest analysis. Regions-of-interest were identified using probabilistic anatomical maps available in SPM Anatomy Toolbox (Eickhoff *et al.*, 2005). Probabilistic anatomical maps were created from microscopic histological *post-mortem* analysis of ten brains. Each map describes the anatomical probability of finding the regions-of-interest at each voxel in MNI reference space, based on the relative frequency of finding the areas in the same space in the ten-brain analysis. Based on the probability maps, each voxel was assigned to the most probably area by applying an algorithm to the probabilistic anatomical maps available in the toolbox, previously described (Eickhoff *et al.*, 2005) (Figure 2). The nucleus basalis of Meynert is located in the basal forebrain, which is composed of cholinergic cell

groups defined histologically as Ch1-Ch6. Ch4 corresponds to the nucleus basalis of Meynert (Mesulam *et al.*, 1983), and was identified using an existing available probabilistic anatomical map (Zaborszky *et al.*, 2008). Similarly, the entorhinal cortex, amygdala, hippocampus, and insula were identified using existing available probabilistic anatomical maps (Kurth *et al.*, 2010). The thalamus was identified using the Thalamic Connectivity atlas by Behrens (Behrens *et al.*, 2003). A reference region was identified as the primary somatosensory cortex area 3a through existing available probabilistic anatomical maps (Choi *et al.*, 2006). This area confirmed anatomical findings, as the primary somatosensory cortex is relatively unaffected in Parkinson's disease and dementia pathology (Burton *et al.*, 2004). Regions-of-interest analysis was performed on normalized MNI space images and repeated on co-registered T1-MRI applying the partial volume correction (Zhang *et al.*, 2016). Potential artefacts due to partial volume were reduced by extracting regions-of-interests in diffusion tensor imaging conditioned on brain tissue content derived from the corresponding segmented structural MRI data. Specifically, to reduce artefacts due to brain atrophy, the regions-of-interests were extracted from regions with more than 90% probability of brain tissue content. To further reduce artefacts to partial brain tissue volume, a threshold of more than 50% probability of grey matter content was applied for regions-of-interests in grey matter areas, as previously done in other PPMI studies (Zhang *et al.*, 2016).

Voxel-based analysis. Voxel-wise statistics for between-group comparisons were computed using appropriately weighted contrasts to localize significant changes in mean voxel values on voxel based morphometry and diffusion tensor imaging fractional anisotropy and mean diffusivity MNI images. The contrasts were used to derive Z-scores on a voxel basis using the general linear model. The threshold for statistical significance was set to

P<0.001 (uncorrected). Voxel-based analysis was performed using SPM12 implemented in Matlab 8.4.

White matter lesion volume. Quantification of white matter lesions was performed by using T1-MRI and T2/FLAIR MRI (Leritz *et al.*, 2014). White matter lesions volumes in T1-MRI were calculated with the Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/), as described previously (Fischl *et al.*, 2002; Fischl *et al.*, 2004). Each subject's T1-MRI lesion mask was overlaid on co-registered T2/FLAIR images for quality control on final volumetric data (Supplementary Methods). In details, the volumetric T1-MRI images were processed to remove non-brain tissue using a hybrid watershed/surface deformation procedure (Segonne *et al.*, 2004), automated Talairach transformation, and segmentation of the subcortical white matter and deep gray matter volumetric structures (Fischl *et al.*, 2002; Fischl *et al.*, 2004). White matter lesions were labelled using a probabilistic procedure (Fischl *et al.*, 2002). Total white matter lesions (hypointensity) volume was then calculated for each hemisphere; these were averaged together to create a single white matter lesions volume for each subject. A manual quality check of the output of the Freesurfer analysis, for each individual MRI, was performed with the freeview software and lesions volume amended accordingly (G.P., J.B.). White matter lesions T1-hypointensity to be labelled in a more restricted portion of tissue compared to hyperintensity volumes measured on T2/FLAIR (Salat *et al.*, 2010). To reduce this bias, quality control was performed on final volumetric data by overlaying each subject's lesion map on the T2/FLAIR image (J.S., G.P.). None of the lesion masks had errors that would require exclusion. There were minor errors, particularly in the determination of the boundaries of large lesions. However, we did not prefer to correct them manually, as the

intra-rater and inter-rater variability associated with manual delineations could potentially bias the results.

Statistical analysis. Statistical analysis was performed in Statistical Package for Social Sciences (SPSS 23.0) software (SPSS Inc., Chicago, IL). Normality was tested with the Shapiro-Wilk test (<50 values) or Kolmogorov-Smirnov test (≥50 values), as appropriate. Continuous variables were expressed as mean and standard deviations in parentheses and compared using independent samples T-Test (normally distributed) and exact Mann-Whitney U test (not normally distributed). Multivariate analysis of variance (MANOVA) was used to assess the main effects of regional structural and microstructural changes among the groups. If the overall multivariate test was significant, *P*-values for each variable were calculated following Bonferroni's multiple comparisons test. Subsequently, MANOVA was repeated adding age as covariate. Categorical variables were expressed as proportions and compared using Fisher's test. To determine predictors of cognitive decline, Cox survival proportional hazards analyses were performed using each regions-of-interest as a predictor of cognitive impairment at univariate analysis. Multivariate Cox survival analyses were carried out including each significant regions-of-interest at univariate and, as covariates, clinical and non-clinical predictors of cognitive impairment previously validated in the PPMI study by Schrag *et al.* (2017). The analyses have been also repeated including as covariates in the model: age, white matter lesions volume and presence of axial motor symptoms. The first occurrence of cognitive impairment at follow up was used as for the time-to-event in the Cox model. To increase stability of our findings, we confirmed that the outcome present at one visit was still present at the subsequent visits. Grey matter mean voxel values were increased by a factor 100, while fractional anisotropy and mean diffusivity diffusion tensor imaging mean voxel values were increased by a factor 1000 in Cox survival analysis. Kaplan–Meier survival estimates were generated after stratifying Parkinson's disease patients by abnormal

regions-of-interest mean voxel values compared using log-rank (Mantel Cox) test. Abnormal regions-of-interest mean voxel was defined as 1 SD from our population of healthy controls. A *P* value of less than 0.05 was used as the cut-off point to be determined a statistically significant result.

Data availability. All data used in this study is available from PPMI database (www.ppmi-info.org/data).

Results

Grey matter changes and cognitive impairment in Parkinson's disease patients. A total of 304 Parkinson's disease patients were identified to be included in this study (Table 1). We first conducted a cross-sectional comparison of grey matter mean voxel values between healthy controls and Parkinson's disease patients at baseline. No differences were found for the nucleus basalis of Meynert, entorhinal cortex, amygdala, hippocampus, insula, thalamus, or primary somatosensory cortex (reference region). After partial volume correction, no differences were found in any regions-of-interest (Table 2). Then, we stratified Parkinson's disease patients into two subgroups: screened as cognitively normal (PD-MoCA≥26, n=232) and screened as cognitively impaired (PD-MoCA≤25, n=72). We found lower grey matter mean voxel values of nucleus basalis of Meynert (P=0.016), amygdala (P=0.022) and thalamus (P=0.001) in cognitively impaired vs. cognitively normal Parkinson's disease patients. No differences were found for other regions-of-interests and the results were consistent when covariate for age. After partial volume correction, we found lower grey matter mean voxel values of nucleus basalis of Meynert (P=0.01) and thalamus (P<0.001) but no differences in the amygdala or other areas (Figure 3A-C, Table 3).

Regions-of-interest-analysis was repeated at voxel-based level and confirmed these results. Statistical parametric maps showed a reduction in grey matter at full brain voxel-based analysis in cognitively impaired compared to cognitively normal Parkinson's disease patients (Supplementary Table 1, Figure 4A).

Microstructural changes and cognitive impairment in Parkinson's disease patients. We conducted a cross-sectional comparison of diffusion tensor imaging fractional anisotropy and mean diffusivity mean voxel values between heathy controls and Parkinson's disease patients on a *priori* regions-of-interest selection at baseline. We found no differences in any fractional anisotropy or mean diffusivity regions-of-interest mean voxel values. These results were confirmed at voxel-based level and after partial volume correction, which showed no differences in any areas (Table 2). Then, we stratified Parkinson's disease patients into two subgroups: screened as cognitively normal (PD-MoCA≥26, n=64) and screened as cognitively impaired (PD-MoCA≤25, n=20). We found increased mean diffusivity mean voxel values of nucleus basalis of Meynert (P=0.03), entorhinal cortex (P=0.02), insula (P=0.02), and thalamus (P=0.04) in cognitively impaired vs. cognitively normal Parkinson's disease patients. No differences were found for other areas. After partial volume correction, we found higher mean diffusivity mean voxel values of the nucleus basalis of Meynert (P=0.045), entorhinal cortex (P=0.002), amygdala (P=0.020), hippocampus (P<0.001), and thalamus (P=0.001) in cognitively impaired vs. cognitively normal Parkinson's disease patients. We also found lower fractional anisotropy mean voxel values of the amygdala (P=0.033), hippocampus (P=0.033), and thalamus (P=0.005) in cognitively impaired vs. cognitively normal Parkinson's disease patients (Figure 3D-G, Table 3).

Regions-of-interest-analysis was repeated at voxel-based level and confirmed these results. Statistical parametric maps showed increased in mean diffusivity at voxel-based analysis in cognitively impaired compared to cognitively normal Parkinson's disease patients (Supplementary Table 1, Figure 4B).

Grey matter changes as predictors of Parkinson's disease cognitive decline. At multivariate Cox survival analysis of regions-of-interests and age as a co-variate, the nucleus basalis of Meynert grey matter mean voxel value was the only statistically significant predictor of developing cognitive impairment (positive Level 2 diagnosis) in Parkinson's disease: [HR]: 0.908, [C.I.]: 0.843–0.978, Wald: 12.067, P=0.001. After partial volume correction, the nucleus basalis of Meynert grey matter mean voxel value remained the only statistically significant predictor of developing cognitive impairment: [HR]: 0.906, [C.I.]: 0.830–0.991, Wald: 11.447, P=0.003 (Table 4).

Since previous studies have suggested that University of Pennsylvania smell identification test (UPSIT), Rapid Eye Movement (REM) Sleep Behavior Disorder Screening Questionnaire (RBDSQ) assessed REM Sleep Behavior Disorder (RBD), Geriatric Depression Scale (GDS), Movement Disorder Society sponsored Unified Parkinson Disease Rating Scale (MDS-UPDRS) Part-III, postural instability, Apo-E genotype group, and Amyloid-β:Tau ratio could be also predictors of cognitive impairment (Muller *et al.*, 2013; Schrag *et al.*, 2017), we carried out further bivariate Cox survival analysis to determine whether nucleus basalis of Meynert grey matter mean voxel value remained a statistically significant predictor of developing cognitive impairment when adjusting for each of these variables as covariates in addition to age. We found that nucleus basalis of Meynert grey matter mean voxel value remained a predictor of cognitive impairment when adjusted for

UPSIT, RBDSQ, GDS, MDS-UPDRS Part-III, Apo-E, and Amyloid-β:Tau, axial gait score, and white matter lesions volume (Supplementary Table 2). We then carried out a multivariate Cox survival analysis with all nine parameters as covariates and further confirmed that nucleus basalis of Meynert grey matter mean voxel value as a statistically significant predictor of developing cognitive impairment: [HR]: 0.919, [C.I.]: 0.870–0.971, Wald: 9.211, P=0.002. As previously indicated by Schrag *et al.* (2017), we also found UPSIT ([HR]: 0.945, [C.I.]: 0.894–0.999, Wald: 4.014, P=0.045) and Amyloid-β:Tau ([HR]: 202.716, [C.I.]: 5.870–7000.656, Wald: 8.640, P=0.003) to be significant predictors of cognitive impairment in the Cox survival analysis with all parameters as co-variates. We further compared these parameters between the patient groups who developed cognitive impairment and those who did not. We found increases in scores amongst patients who developed cognitive impairment in UPSIT, RBDQS, GDS, MDS-UPDRS Part-III, Amyloid-β:Tau ratio, and axial gait score. There were no differences in Apo-E genotype or white matter lesions volume (Supplementary Table 3).

Multivariate Cox survival with the same nine parameters was carried out after partial volume correction and showed the nucleus basalis of Meynert grey matter mean voxel value as a significant predictor of cognitive impairment: [HR]: 0.928, [C.I.]: 0.865–0.995, Wald: 4.436, P=0.035. Similarly, we also found UPSIT ([HR]: 1.175, [C.I.]: 1.007–1.372, Wald: 4.189, P=0.041) and Amyloid-β:Tau ([HR]: 78.317, [C.I.]: 2.501–2452.692, Wald: 6.158, P=0.013) to be predictors of cognitive impairment.

We stratified patients using the average of nucleus basalis of Meynert grey matter mean voxel values within heathy controls minus one standard deviation (0.278 mean voxel value) and generated Kaplan-Meier estimates (Figure 3H). The groups had significantly different

cognitive impairment-free survival times (X^2=8.78, *P*=0.003, 1 degree of freedom) when compared by a log rank (Mantel-Cox) test.

Microstructural changes as predictors of Parkinson's disease cognitive decline. At multivariate Cox survival analysis of mean diffusivity regions-of-interests and age as a co-variate, we found the nucleus basalis of Meynert mean voxel value ([HR]: 319.587, [C.I.]: 6.830-14954.816, Wald: 8.638, *P*=0.003) and entorhinal cortex ([HR]: 0.015, [C.I.]: 0.001-0.942, Wald: 3.953, *P*=0.047) mean voxel value to be statistically significant predictors of developing cognitive impairment (Table 4). We then carried out a multivariate Cox survival analysis with all nine parameters as covariates and further confirmed nucleus basalis of Meynert mean diffusivity mean voxel value as a statistically significant predictor of developing cognitive impairment: [HR]: 116.445, [C.I.]: 1.085–12497.762, Wald: 3.977, *P*=0.046. We carried out further bivariate Cox survival analysis to determine whether nucleus basalis of Meynert mean diffusivity mean voxel value remained a statistically significant predictor of developing cognitive impairment when adjusting for each clinical variables and biomarkers as covariates (in addition to age). We found that nucleus basalis of Meynert mean diffusivity mean voxel value remained a predictor of cognitive impairment when adjusted for UPSIT, RBDSQ, GDS, MDS-UPDRS Part-III, Apo-E, and Amyloid-β:Tau, axial gait, and white matter lesions volume (Supplementary Table 2). After partial volume correction, at multivariate Cox survival analysis the nucleus basalis of Meynert mean diffusivity mean voxel value was the only significant predictor of cognitive impairment: [HR]: 11.371, [C.I.]: 1.025-126.172, Wald: 3.920, *P*=0.048 (Table 4). At multivariate Cox survival analysis of fractional anisotropy regions-of-interest, no areas were statistically significant predictors of developing cognitive impairment (positive Level 2 diagnosis) in Parkinson's disease.

We stratified patients using the healthy control mean nucleus basalis of Meynert diffusion tensor imaging mean diffusivity mean voxel values plus one standard deviation (0.00115 mean voxel value) and generated Kaplan-Meier estimates (Figure 3I). The groups had significantly different cognitive impairment-free survival times (X^2=8.03, *P*=0.005, 1 degree of freedom) when compared by a log rank (Mantel-Cox) test.

Comparison of predictive value between grey and microstructural white matter data. To identify the better predictor between structural and microstructural changes within the nucleus basalis of Meynert, we performed a bivariate Cox survival including both mean diffusivity and grey matter nucleus basalis of Meynert. We found mean diffusivity to be a statistically significant predictor of cognitive impairment ([HR]: 72.73, [C.I.]: 1.916-2760.399, Wald: 5.338, *P*=0.021), but not grey matter ([HR]: 0.941, [C.I.]: 0.881-1.006, Wald: 3.200, *P*=0.074).

Axial symptoms and cognitive impairment. We further investigated whether axial gait is associated with cognitive decline in our population of drug-naïve Parkinson's disease patients. In the cross-sectional baseline analysis, we found no difference in axial gait score (*P*=0.21). However, at follow-up analysis, cognitively normal Parkinson's disease patients who developed cognitive impairment had a higher axial gait score (*P*=0.02, Supplementary Table 3). These results were stable after including age as a co-variate in the Cox survival analysis (HR: 3.144, C.I.: 1.007-9.810, Wald: 3.892, *P*=0.049). However, in a multivariate Cox survival analysis including the axial gait score with grey matter nucleus basalis of Meynert and all nine parameters as covariates, the axial gait score lose its power prediction: [HR]: 2.255, [C.I.]: 0.493–10.316, Wald: 1.099, P=0.294).

White matter lesions volume and cognitive impairment. We investigated whether white matter lesions volume is associated with cognitive decline. We found no difference in white matter lesions volume between heathy controls and Parkinson's disease patients (t=0.416, *P*=0.68). In cognitively impaired Parkinson's disease patients, we found a trend (not significant) of increased white matter lesions volume compared to cognitively normal patients (2815.4±2983.7 in PD-MoCA≥26 vs. 3410.9±2980.6 in PD-MoCA≤25; t=-1.48, *P*=0.14). At follow-up, cognitively normal Parkinson's disease patients who developed cognitive impairment did have only a trend (not significant) of higher white matter lesions volume (t=-1.51 *P*=0.14, Supplementary Table 3) compared to patients who did not develop cognitive impairment. In a Cox survival analysis, we found white matter lesions volume to be a predictor of developing cognitive impairment in Parkinson's disease: [HR]: 1.000, [C.I.]: 1.000-1.000, Wald: 6.643, *P*=0.01. However, when the Cox survival analysis was adjusted for age as a co-variate, white matter lesions volume lose its power of prediction [HR]: 1.000, [C.I.]: 1.000-1.000, Wald: 0.821, *P*=0.365.

Assessment of variability between MRI scanners. No regions-of-interest differences in grey matter mean voxel values were found between T1-MRI data obtained by different manufacturers (Philips vs. GE. vs. SIEMENS) or strength of field (1.5 vs. 3T). No regions-of-interest differences in mean diffusivity mean voxel values were found between diffusion tensor imaging data obtained by different diffusion tensor imaging protocols (gated vs. non-gated), or acquired at different centres (Supplementary Table 4 and 5).

Discussion

In this study, we set out to prove a hypothesis of a significant relationship between the damage of cholinergic system and the development of cognitive impairment in Parkinson's disease, and demonstrated that microstructural damages of the nucleus basalis of Meynert underlines and predicts clinical onset of cognitive impairment in Parkinson's disease.

We investigated structural and microstructural changes in several brain regions related to cholinergic system and associated limbic pathways in cognitively impaired compared to cognitively intact patients with Parkinson's disease, and relatively to a group of age-matched heathy controls. We also followed-up cognitively intact Parkinson's disease patients for 36-months to identify early predictors of cognitive impairment. We used MoCA as a screening tool for cognitive impairment at baseline, and Level 2 diagnosis also including self-reported issues in cognitive function, and impairment on at least two cognitive domains at follow-up. A more sensitive screening tool was beneficial at baseline to look for predictors of cognitive impairment in all patients with current cognitive impairment or in early developmental stages of cognitive impairment. Additionally, when looking for predictors of cognitive impairment in a longitudinal design, we wanted to ensure that predictors were identified before the onset of clinical cognitive impairment. Therefore, we aimed to exclude all patients who had early detectable stages of cognitive impairment using this more sensitive test.

Cross sectional comparison between Parkinson's disease patients and heathy controls revealed no differences in grey matter or diffusion tensor imaging mean diffusivity or fractional anisotropy. Separate subcortical volumetric and microstructural differences were, however, identified between cognitively normal and cognitively impaired Parkinson's disease patients. We found loss of grey matter and increased diffusion tensor imaging mean diffusivity in the nucleus basalis of Meynert and thalamus of Parkinson's disease patients

with cognitive impairment, which indicates damage in these structures. The involvement of the nucleus basalis of Meynert in cognitive decline in Parkinson's disease has been described previously (Gratwicke *et al.*, 2015). Additionally, here, decreased thalamic volume was observed in Parkinson's disease patients with cognitive impairment, as suggested by Chen *et al.* (2016) who closely associated early cognitive decline in Parkinson's disease with the atrophy of the thalamus. These findings demonstrate that changes in both structural (reduced grey matter voxel mean) and microstructural (increased mean diffusivity) levels in cholinergic structures underline cognitive impairment in patients with Parkinson's disease. Our results were confirmed with region-of-interest and voxel-based analyses, and after correction for partial volume effects. Interestingly, we found that cognitively impaired patients had microstructural changes also in the entorhinal cortex (increased mean diffusivity), but not changes in structural grey matter voxel-based morphometry. The entorhinal cortex has prominent cholinergic innervation (Heys *et al.*, 2012), and histopathology has demonstrated that is also affected early by tau pathology (Braak *et al.*, 2006).

Our longitudinal findings indicated that structural and microstructural changes in the nucleus basalis of Meynert were predictive for developing cognitive impairment in patients with Parkinson's disease. Degeneration of the nucleus basalis of Meynert occurs before the onset of cognitive impairment, or while cognitive impairment is subclinical. Using Cox survival analysis, we established that the HR for developing cognitive impairment increases by 9.2% per every 0.01 decrease in grey matter mean voxel value in the nucleus basalis of Meynert. We also established that the HR for developing cognitive impairment increases by a factor of 11.4 per every 0.001 increase in diffusion tensor imaging diffusivity mean voxel value in the nucleus basalis of Meynert. Several studies have suggested indicators of cognitive

impairment in Parkinson's disease, and age is widely recognised as a risk factor of cognitive impairment in both Parkinson's disease patients and in the general population (Williams-Gray *et al.,* 2013). After adjusting for age in addition to other clinical and non-clinical indicators of cognitive impairment suggested by Schrag *et al.* (2017), damage in nucleus basalis of Meynert remained as a statistically significant indicator of cognitive impairment. Thereby, we provide further evidence that the degeneration of the nucleus basalis of Meynert precedes and predicts the onset of cognitive impairment, independently to other clinical and non-clinical markers of Parkinson's disease.

Moreover, our findings show that if both grey matter voxel mean and mean diffusivity of nucleus basalis of Meynert were included in the same Cox model, mean diffusivity remained the only predictor of cognitive impairment. This suggests that microstructural changes in the nucleus basalis of Meynert may precede the structural damage of grey matter measured with voxel-based morphometry. This is in line with the finding that cognitively impaired patients at baseline had increased mean diffusivity but normal grey matter voxel mean within the entorhinal cortex, which is the area with greater connections with nucleus basalis of Meynert.

Our findings, however, do not indicate when the degeneration of nucleus basalis of Meynert starts, and how the onset of degeneration relates to the onset of cognitive impairment. Braak *et al.* (2003) suggested that Lewy body accumulation of cholinergic neurons in the basal forebrain occurs at the same stage as the degeneration of dopaminergic neurons in the substantia nigra pars compacta. This is in line with the finding of Burciu and colleagues (2017), who found microstructural changes measured in the substantia nigra are key predictors of motor progression in Parkinson's disease (Burciu *et al.*, 2017). Taken together our study with that of Burciu, is possible that measuring the pathological processes at

microstructural levels with diffusion tensor imaging may be a reliable tool to predict the development of cognitive decline (measuring the damage of the nucleus basalis of Meynert) and motor progression (measuring the damage of the substantia nigra) in the early stages of Parkinson's disease. Future studies need to clarify this combined predictive value of diffusion tensor imaging in the same individuals.

Previous studies have also suggested that white matter lesions are associated with cortical cholinergic deafferentation in elderly with leukoaraiosis (Bohnen *et al.*, 2009b). White matter lesions at the frontal horns are in close proximity to cholinergic axons that originate in the nucleus basalis of Meynert (Bohnen *et al.*, 2009a). Therefore, these lesions may result in more significant cortical deafferentation because of the more proximal axonal disruption (Bohnen *et al.*, 2009a). We investigated the presence of white matter lesions (combining T1 and T2-weighted images) and found no differences in cognitively impaired compared to cognitively normal Parkinson's disease patients at baseline. In our population, white matter lesions longitudinally predicted the development of cognitive impairment. However, when corrected for age, white matter lesions lost their power of prediction, which suggests that the ageing process more than white matter lesions are associated with cognitive decline. White matter lesions have also been associated with gait dysfunction, another symptom of Parkinson's disease probably related to the damage of cholinergic system (Bohnen and Albin, 2011b). The presence of gait dysfunction and the degree of axial symptoms have been associated with the development of cognitive impairment in Parkinson's disease (Bohnen *et al.*, 2009a; Bohnen and Albin, 2011b; Muller *et al.*, 2013). We investigated the degree of axial involvement in our population of early Parkinson's disease patients. We found that cognitively impaired patients had greater axial gait symptoms compared to cognitively normal Parkinson's disease patients at baseline. Axial gait symptoms were predictive of

cognitive decline when considered alone, but their power as predictors was lower than diffusion tensor imaging or voxel-based morphometry. Morever, in the full prognostic model including all the known predictors of cognitive impairment, axial gait symptoms lost their power of prediction. This may suggest that, in Parkinson's patients with axial symptoms, the degeneration of cholinergic nuclei involves not only the pedunculopontine nucleus (associated with gait dysfunction) but also the nucleus basalis of Meynert. Thus, a damage of nucleus basalis of Meynert might be a common mediator of the development of cognitive impairment explaining why most of the studies (that not measured the damage of nucleus basalis of Meynert) found an association between gait and cognitive decline.

Our research provides a realistic, cost effective and non-invasive way to identify Parkinson's disease patients at higher risk to develop cognitive impairment, before clinical symptomatic onset. This represents and unmet need; the opportunity to evaluate only one reliable predictor of cognitive impairment in common clinical practice to allow us to assess patients and stratify their risk of prodromal cognitive impairment at early stages of the disease, improving patient care and outcomes(Anang *et al.*, 2017). We provide a clinical tool to screen people in a routine clinical MRI. We propose that this can further be used by clinicians to assess sub-phenotypes of Parkinson's disease at higher risk of cognitive impairment as well as to investigate cognitive impairment and non-motor symptom progression. Clinical trials may then be tailored on patients at high risk of cognitive impairment, increasing the power of the analysis for the identification of disease modification treatments.

In identifying regions-of-interests we used combined probabilistic maps available in Anatomy Toolbox, a relatively new method. We believe Anatomy Toolbox serves to provide more accurate regions-of-interest parameters than conventional brain atlases without having

to manually identify regions and correct for errors. Probabilistic maps accounts for individuals' structural differences whilst utilising a standardised mapping format. Additionally, we acknowledged a limitation of this study to be the difficulty in identifying cognitive impairment as a prodromal form of Parkinson's disease dementia, rather than another form of dementia. To account for this, we distinguished between developing dementia after Parkinson's disease, Parkinson's disease and dementia concurrently, and dementia preceding Parkinson's disease by excluding patients screened as cognitively impaired at baseline for follow up study. This removed patients with dementia preceding Parkinson's disease, and Parkinson's disease and dementia concurrently, which may be caused by dementia with Lewy Bodies (Bohnen and Albin, 2011a). However, the only conclusive way of confirming cognitive impairment in Parkinson's disease as a prodromal stage of dementia is with a histological *post-mortem* exam.

In conclusion, we demonstrated here: (a) that nucleus basalis of Meynert is a predictor of cognitive impairment in a population of early drug-naïve Parkinson's patients; (b) that microstructural changes are stronger predictors compared to structural changes, even after partial volume correction; (c) that structural changes at voxel-based morphometry and microstructural changes at diffusion tensor imaging are significant predictor of cognitive decline also in a model including all the previously suggested predictors (from other studies) of cognitive impairment in Parkinson's disease; (d) that white matter lesions are predictors of cognitive impairment only when ageing in not accounted. As the prevalence of Parkinson's disease increases exponentially with age and prevalence of cognitive impairment increases alongside with the evolution of Parkinson's disease, a reliable biomarker to identify those patients at higher risk for cognitive impairment is now more important than ever.

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Figure Legends

Figure 1 | Study population. Subjects identified for study (**A**) Heathy controls and Parkinson's disease patients included for grey matter analysis (**B**) Heathy controls and Parkinson's disease patients included for diffusion tensor imaging analysis as fractional anisotropy and mean diffusivity changes. CI: cognitively impaired; MoCA: Montreal Cognitive Assessment Scale.

Figure 2 | A *priori* regions-of-interests. Anatomy Toolbox regions-of-interests in Montreal Neurological Institute (MNI) space displayed in coronal (left column) and sagittal (right column) views. (**A**) nucleus basalis of Meynert (**B**) entorhinal cortex (**C**) amygdala (**D**) hippocampus (**E**) insula (**F**) thalamus (**G**) primary somatosensory cortex.

Figure 3 | (A)–(G) Regions-of-interest grey matter volume of Parkinson's disease patients screened at baseline for cognitive function. Patients stratified by cognitive function using MoCA test (Level 1 diagnosis, Methods). Cognitively normal defined as MoCA≥25 n=232, cognitively impaired defined as MoCA≤25 n=72. (A)-(G) Individual grey matter regions-of-interests. Median, 10th, 25th, 75th, and 90th percentile of regions-of-interest mean voxel value shown as box plot. Groups compared by independent samples t-tests, with 302 degrees of freedom (equal variance assumed). Equality of variance tested by Levene's test. Statistically significant results indicated by P-values: *P<0.05, **P<0.01. **(H) Cumulative cognitive impairment-free (CI-free) progression amongst Parkinson's disease patients stratified by grey matter nucleus basalis of Meynert volume.** 232 Parkinson's disease patients screened as cognitively normal at baseline, at follow up for up to 36 months 35 patients developed clinically confirmed CI (PD-CI) and 197 patients remained

cognitively normal (PD-noCI). Patients stratified by a split of heathy control mean nucleus basalis of Meynert grey matter (GM) mean voxel values minus one standard deviation (*) and Kaplan-Meier graph generated. Log rank (Mantel-Cox) test indicates cumulative development of cognitive impairment over 36 months are statistically different (X^2=8.78, *P*=0.003, 1 degree of freedom). **(I) Cumulative cognitive impairment-free (CI-free) progression amongst Parkinson's disease patients stratified by diffusion tensor imaging mean diffusivity of the** nucleus basalis of Meynert**.** 34 Parkinson's disease patients screened as cognitively normal at baseline, at follow up for up to 36 months 17 patients developed clinically confirmed cognitive impairment (PD-CI) and 47 patients remained cognitively normal (PD-noCI). Patients were stratified by a split of heathy control mean nucleus basalis of Meynert mean diffusivity (MD) mean voxel values plus one standard deviation (*) and Kaplan-Meier graph generated. Log rank (Mantel-Cox) test indicates cumulative development of cognitive impairment over 36 months are statistically different (X^2=8.03, *P*=0.005, 1 degree of freedom).

Figure 4 | (A) Statistical parametric maps showing reduction in grey matter at voxel-based analysis cognitively impaired compared to cognitively normal Parkinson's disease patients (MNI co-ordinates Nucleus basalis of Meynert, right: x = -4, y = -3, z = -9, left: x = -6, y = -1, z = -9, Thalamus, right: x = 2, y = -13, z = 7, left: x = -2, y = -13, z = 7 and Amygdala right: x = -30, y = -111, z = -23, left: x = 30, y = -9, z = -23). Yellow–red areas represent voxel clusters with decreases values within the full brain. P<0.001 uncorr. The colour stripe indicates z-values. **(B)** Statistical parametric maps showing increased in mean diffusivity at voxel-based analysis in cognitively impaired compared to cognitively normal Parkinson's disease patients (MNI co-ordinates Nucleus basalis of Meynert, right: x = -6, y = -4, z = -8, left: x = 6, y = -2, z = -7, Thalamus, right: x = -5, y = -9, z = 1, left: x = 12, y = -21, z = 4,

Entorhinal cortex, right: x = -21, y = 4, z = -27, left: x = 23, y = 7, z = -26, and Insula, right: x = -51, y = -17, z = -5, left: x = 48, y = -4, z = -16**).** Yellow–red areas represent voxel clusters with increased in MD values within the full brain. P<0.001 uncorr. The colour stripe indicates z-values.

Tables

Table 1. Baseline demographics and clinical characteristics

	Study groups		
	Heathy controls	**Parkinson's disease patients**	**t-test**
Demographic features			
Sex (female, male)	167 (57, 110)	304 (104, 200)	t=0.02 *P*=0.99
Age	59.9 (11.4)	61.4 (9.5)	t=-1.49 *P*=0.14
Age of onset	-	60.9 (9.5)	-
Duration of disease	-	6.6 (6.6)	-
PD family history, positive %	4.3	25.3	t=-7.12 *P*<0.0001
Education	16.1 (2.8)	15.5 (2.9)	t=2.02 *P*=0.04
Clinical characteristics			
MDS-UPDRS Part-I	0.6 (1.1)	1.2 (1.5)	t=-5.10 *P*<0.0001
MDS-UPDRS Part-I Quest.	2.4 (2.5)	4.3 (3.1)	t=-7.12 *P*<0.0001
MDS-UPDRS Part-II	0.4 (0.9)	5.9 (4.2)	t=-21.73 *P*<0.0001
MDS-UPDRS Part-III	1.3 (2.7)	20.9 (9.1)	t=-34.84 *P*<0.0001
H&Y	0.0 (0.1)	1.6 (0.5)	t=-50.79 *P*<0.0001
White matter volume	3107.3 (4837.3)	2956.9 (2988.8)	t=0.42 *P*=0.68
Non-motor symptom status			
GDS	1.4 (2.2)	2.3 (2.4)	t=-4.20 *P*<0.0001
SCOPA-AUT	5.7 (3.8)	9.5 (6.2)	t=-8.17 *P*<0.0001
ESS	5.7 (3.5)	5.7 (3.4)	t=-0.13 *P*=0.90
RBDQS	2.9 (2.3)	4.2 (2.7)	t=-5.09 *P*<0.0001
UPSIT	34.1 (4.7)	22.3 (8.3)	t=19.80 *P*<0.0001
Cognitive status			
MoCA	28.3 (1.1)	27.0 (2.2)	t=7.84 *P*<0.0001
Semantic Fluency Test	51.6 (11.6)	48.2 (10.7)	t=3.15 *P*=0.002
HVLT Immediate Recall	26.0 (4.5)	24.4 (5.0)	t=3.42 *P*=0.001
SDM	47.6 (10.7)	41.7 (9.8)	t=5.98 *P*<0.0001
Benton JLO	13.2 (2.0)	12.9 (2.1)	t=1.77 *P*=0.008

MDS-UPDRS: Movement Disorder Society sponsored Unified Parkinson Disease Rating Scale (Part-I, Part-I Questionnaire, Part-II, and Part-III); H&Y: Hoehn & Yahr scale; GDS: 15-item Geriatric Depression Scale; SCOPA-AUT: Scales for Outcomes in Parkinson's disease - Autonomic; ESS: Epworth Sleepiness Scale; RBDQS: REM Sleep Behavior Disorder Screening Questionnaire; UPSIT: University of Pennsylvania Smell Identification Test; MoCA: Montreal Cognitive Assessment Scale; HVLT: Hopkin's Learning Verbal Test; SDMT Symbol Digit Modalities Test; JLO: Benton Judgement of Line Orientation Test. Tabled values are the mean of each group with standard deviation in parenthesis (unless indicated otherwise). Age, age of onset, and education measured in years, duration of disease measured in months.

Table 2. Baseline Grey matter and diffusion tensor imaging regions-of-interest volumes between Heathy controls and Parkinson's disease patients

	Study groups (mean voxel value)		
	Heathy controls	**Parkinson's disease patients**	**t-test**
Grey matter regions-of-interest volumes			
Nucleus basalis of Meynert	0.361 (0.083)	0.357 (0.089)	t=0.39 P=0.69
Entorhinal cortex	0.530 (0.075)	0.526 (0.081)	t=0.54 P=0.59
Amygdala	0.587 (0.063)	0.587 (0.065)	t=-0.08 P=0.94
Hippocampus	0.492 (0.055)	0.496 (0.059)	t=-0.78 P=0.44
Insula	0.367 (0.056)	0.367 (0.057)	t=-0.09 P=0.93
Thalamus	0.234 (0.035)	0.234 (0.034)	t=-0.04 P=0.97
Primary somatosensory cortex	0.194 (0.050)	0.190 (0.048)	t=0.77 P=0.44
Grey matter regions-of-interest volumes after partial volume correction			
Nucleus basalis of Meynert	0.397 (0.060)	0.392 (0.061)	t=0.82 P=0.41
EC	0.515 (0.062)	0.514 (0.062)	t=0.13 P=0.90
Amygdala	0.514 (0.055)	0.514 (0.055)	t=0.07 P=0.95
Hippocampus	0.449 (0.053)	0.453 (0.053)	t=-0.70 P=0.49
Insula	0.472 (0.057)	0.474 (0.054)	t=-0.31 P=0.75
Thalamus	0.193 (0.028)	0.191 (0.027)	t=0.70 P=0.48
Primary somatosensory cortex	0.291 (0.053)	0.290 (0.050)	t=0.16 P=0.87
Diffusion tensor imaging regions-of-interest fractional anisotropy and mean diffusivity			
Fractional anisotropy			
Nucleus basalis of Meynert	0.450 (0.035)	0.449 (0.034)	t=0.13 P=0.89
Entorhinal cortex	0.203 (0.017)	0.205 (0.019)	t=-0.64 P=0.53
Amygdala	0.237 (0.018)	0.237 (0.019)	t=-0.03 P=0.98
Hippocampus	0.209 (0.021)	0.210 (0.020)	t=-0.11 P=0.91
Insula	0.223 (0.016)	0.222 (0.014)	t=0.34 P=0.73
Thalamus	0.313 (0.030)	0.312 (0.025)	t=0.08 P=0.94
Primary somatosensory cortex	0.151 (0.017)	0.152 (0.017)	t=-0.34 P=0.73
Mean diffusivity*100			
Nucleus basalis of Meynert	0.124 (0.016)	0.124 (0.015)	t=-0.18 P=0.86
Entorhinal cortex	0.118 (0.020)	0.118 (0.014)	t=0.10 P=0.92
Amygdala	0.092 (0.009)	0.092 (0.009)	t=0.14 P=0.89
Hippocampus	0.113 (0.017)	0.115 (0.021)	t=-0.54 P=0.59
Insula	0.106 (0.012)	0.108 (0.015)	t=-0.70 P=0.49
Thalamus	0.096 (0.015)	0.096 (0.017)	t=0.01 P=0.99
Primary somatosensory cortex	0.105 (0.011)	0.105 (0.011)	t=-0.11 P=0.92
Diffusion tensor imaging regions-of-interest fractional anisotropy and mean diffusivity after partial volume correction			
Fractional anisotropy			
Nucleus basalis of Meynert	0.236 (0.052)	0.236 (0.051)	t=0.05 P=0.96
Entorhinal cortex	0.173 (0.024)	0.170 (0.019)	t=0.69 P=0.49
Amygdala	0.219 (0.031)	0.215 (0.025)	t=0.72 P=0.47

Hippocampus	0.253 (0.042)	0.250 (0.040)	t=0.34 P=0.73
Insula	0.220 (0.033)	0.221 (0.027)	t=-0.17 P=0.86
Thalamus	0.369 (0.033)	0.373 (0.034)	t=-0.68 P=0.49
Primary somatosensory cortex	0.081 (0.028)	0.084 (0.024)	t=-0.81 P=0.42
Mean diffusivity*100			
Nucleus basalis of Meynert	0.128 (0.027)	0.130 (0.030)	t=-0.32 P=0.75
EC	0.106 (0.013)	0.108 (0.012)	t=-1.12 P=0.26
Amygdala	0.107 (0.012)	0.109 (0.012)	t=-1.05 P=0.30
Hippocampus	0.112 (0.015)	0.112 (0.017)	t=-0.26 P=0.79
Insula	0.108 (0.011)	0.112 (0.015)	t=-1.69 P=0.09
Thalamus	0.123 (0.019)	0.123 (0.020)	t=0.01 P=0.99
Primary somatosensory cortex	0.079 (0.027)	0.084 (0.028)	t=-1.24 P=0.22

1 Tabled values are the mean voxel value of each regions-of-interest with standard deviation in parenthesis.

2

Table 3. Baseline Grey matter and diffusion tensor imaging regions-of-interest volumes between cognitively intact and cognitively impaired Parkinson's disease patients

Grey matter regions-of-interest volumes Parkinson's disease patients			
	Parkinson's disease subgroups (mean voxel value)		
	PD-MoCA≥26	**PD-MoCA≤25**	**t-test**
Nucleus basalis of Meynert	0.364 (0.087)	0.335 (0.093)	t=2.43 P=0.02
Entorhinal cortex	0.530 (0.077)	0.515 (0.093)	t=1.34 P=0.18
Amygdala	0.592 (0.064)	0.572 (0.067)	t=2.30 P=0.02
Hippocampus	0.500 (0.056)	0.484 (0.065)	t=1.98 P=0.05
Insula	0.370 (0.057)	0.357 (0.056)	t=1.79 P=0.07
Thalamus	0.238 (0.032)	0.223 (0.036)	t=3.23 P=0.001
Primary somatosensory cortex	0.192 (0.048)	0.184 (0.047)	t=1.31 P=0.19
Grey matter regions-of-interest volumes in Parkinson's disease patients after partial volume correction			
Nucleus basalis of Meynert	0.396 (0.063)	0.377 (0.048)	t=2.26 P=0.01
Entorhinal cortex	0.518 (0.061)	0.503 (0.061)	t=1.76 P=0.08
Amygdala	0.517 (0.055)	0.502 (0.055)	t=1.89 P=0.06
Hippocampus	0.455 (0.052)	0.444 (0.055)	t=1.61 P=0.11
Insula	0.477 (0.055)	0.464 (0.052)	t=1.65 P=0.10
Thalamus	0.194 (0.026)	0.180 (0.028)	t=3.74 P<0.001
Primary somatosensory cortex	0.293 (0.049)	0.280 (0.051)	t=1.96 P=0.05
Diffusion tensor imaging regions-of-interest fractional anisotropy and mean diffusivity in Parkinson's disease patients			
Fractional anisotropy			
Nucleus basalis of Meynert	0.451 (0.034)	0.444 (0.035)	t=0.83 P=0.41
Entorhinal cortex	0.205 (0.020)	0.203 (0.017)	t=0.52 P=0.61
Amygdala	0.239 (0.020)	0.233 (0.017)	t=1.07 P=0.29
Hippocampus	0.210 (0.021)	0.207 (0.017)	t=0.71 P=0.48
Insula	0.224 (0.014)	0.219 (0.012)	t=1.37 P=0.18
Thalamus	0.314 (0.026)	0.307 (0.024)	t=1.05 P=0.30
Primary somatosensory cortex	0.153 (0.018)	0.149 (0.016)	t=0.85 P=0.40
Mean diffusivity*100			
Nucleus basalis of Meynert	0.122 (0.013)	0.132 (0.019)	t=-2.30 P=0.03
Entorhinal cortex	0.116 (0.013)	0.125 (0.016)	t=0.48 P=0.02
Amygdala	0.091 (0.007)	0.096 (0.013)	t=-1.60 P=0.12
Hippocampus	0.111 (0.014)	0.127 (0.032)	t=-2.06 P=0.05
Insula	0.106 (0.013)	0.115 (0.019)	t=-2.34 P=0.02
Thalamus	0.093 (0.013)	0.106 (0.024)	t=-2.23 P=0.04
Primary somatosensory cortex	0.104 (0.011)	0.109 (0.013)	t=-1.53 P=0.13
Diffusion tensor imaging regions-of-interest fractional anisotropy and mean diffusivity in Parkinson's disease patients after partial volume correction			
Fractional anisotropy			
Nucleus basalis of Meynert	0.235 (0.048)	0.237 (0.061)	t=-0.16 P=0.87
Entorhinal cortex	0.172 (0.019)	0.165 (0.020)	t=1.36 P=0.18
Amygdala	0.219 (0.023)	0.205 (0.028)	t=2.17 P=0.03
Hippocampus	0.256 (0.038)	0.234 (0.044)	t=2.17 P=0.03

Insula	0.218 (0.025)	0.231 (0.030)	t=-1.98 P=0.05
Thalamus	0.379 (0.030)	0.354 (0.042)	t=2.91 P=0.005
Primary somatosensory cortex	0.083 (0.024)	0.088 (0.025)	t=-0.87 P=0.38
Mean diffusivity*100			
Nucleus basalis of Meynert	0.125 (0.026)	0.144 (0.037)	t=-2.11 P=0.045
Entorhinal cortex	0.106 (0.011)	0.115 (0.015)	t=-3.20 P=0.002
Amygdala	0.106 (0.007)	0.117 (0.018)	t=-2.52 P=0.02
Hippocampus	0.109 (0.014)	0.124 (0.021)	t=-3.68 P<0.001
Insula	0.110 (0.015)	0.115 (0.016)	t=-1.29 P=0.20
Thalamus	0.119 (0.016)	0.135 (0.026)	t=-3.38 P=0.001
Primary somatosensory cortex	0.082 (0.026)	0.091 (0.032)	t=-1.15 P=0.25

Tabled values are the mean voxel value of each regions-of-interest with standard deviation in parenthesis. MoCA: Montreal Cognitive Assessment Scale.

Table 4. Grey matter and diffusion tensor imaging Predictors of cognitive impairment in Parkinson's disease

		95% C.I.		
	HR	**Lower**	**Upper**	**Sig.**
Grey matter predictors of cognitive impairment in Parkinson's disease				
Nucleus basalis of Meynert	0.908	0.843	0.978	P=0.01
Entorhinal cortex	0.939	0.869	1.015	P=0.12
Amygdala	1.000	0.871	1.148	P=1.00
Hippocampus	1.081	0.933	1.251	P=0.30
Insula	1.029	0.933	1.135	P=0.57
Thalamus	1.011	0.893	1.146	P=0.86
Grey matter predictors of cognitive impairment in Parkinson's disease after partial volume correction				
Nucleus basalis of Meynert	0.907	0.830	0.991	*P*=0.003
Entorhinal cortex	0.972	0.882	1.071	*P*=0.56
Amygdala	1.055	0.885	1.259	*P*=0.55
Hippocampus	1.051	0.884	1.249	*P*=0.58
Insula	0.955	0.847	1.077	*P*=0.45
Thalamus	1.012	0.850	1.204	*P*=0.89
Diffusion tensor imaging predictors of cognitive impairment in Parkinson's disease				
Fractional anisotropy				
Nucleus basalis of Meynert	1.003	0.985	1.021	P=0.77
Entorhinal cortex	1.006	0.985	1.028	P=0.56
Amygdala	1.002	0.951	1.056	P=0.94
Hippocampus	0.986	0.958	1.014	P=0.33
Insula	0.990	0.943	1.038	P=0.67
Thalamus	1.010	0.982	1.039	P=0.50
Mean diffusivity				
Nucleus basalis of Meynert	319.587	6.830	14954.816	P=0.003
Entorhinal cortex	0.015	0.001	0.942	P=0.047
Amygdala	7.839	0.002	32627.914	P=0.63
Hippocampus	5.294	0.158	177.350	P=0.35
Insula	17.892	0.257	1245.817	P=0.18
Thalamus	1.042	0.013	84.864	P=0.99
Diffusion tensor imaging predictors of cognitive impairment in Parkinson's disease after partial volume correction				
Fractional anisotropy				
Nucleus basalis of Meynert	1.008	0.992	1.024	*P*=0.33
Entorhinal cortex	1.017	0.977	1.058	*P*=0.41
Amygdala	0.964	0.914	1.016	*P*=0.17
Hippocampus	1.007	0.981	1.034	*P*=0.59
Insula	1.003	0.987	1.019	*P*=0.75
Thalamus	0.987	0.957	1.018	*P*=0.40
Mean diffusivity				
Nucleus basalis of Meynert	11.371	1.025	126.172	*P*=0.048

Entorhinal cortex	1.499	0.004	503.138	*P*=0.89
Amygdala	3.554	<0.001	409.879	*P*=0.83
Hippocampus	0.197	<0.001	87.934	*P*=0.60
Insula	0.959	0.009	97.507	*P*=0.99
Thalamus	1.250	0.002	842.332	*P*=0.95

Cox survival proportional hazards analysis of grey matter regions-of-interests mean voxel values. Cox survival up to 36 months follow up of 232 Parkinson's disease patients (screened at baseline as cognitively normal): 35 patients developed clinically confirmed cognitive impairment (PD-CI) and 197 patients remained cognitively normal (PD-noCI). Cox survival proportional hazards analysis of diffusion tensor imaging fractional anisotropy and mean diffusivity regions-of-interests mean voxel values. Cox survival (backwards: conditional) up to 36 months follow up of 64 Parkinson's disease patients (screened at baseline as cognitively normal): 17 patients developed clinically confirmed cognitive impairment (PD-CI) and 47 patients remained cognitively normal (PD-noCI). Hazard ratios (HR) produced with 95% confidence interval (C.I.) and statistical significance (Sig.). Age is included as a co-variate.